SIXTH EDITION

ESSENTIAL IMMUNOLOGY

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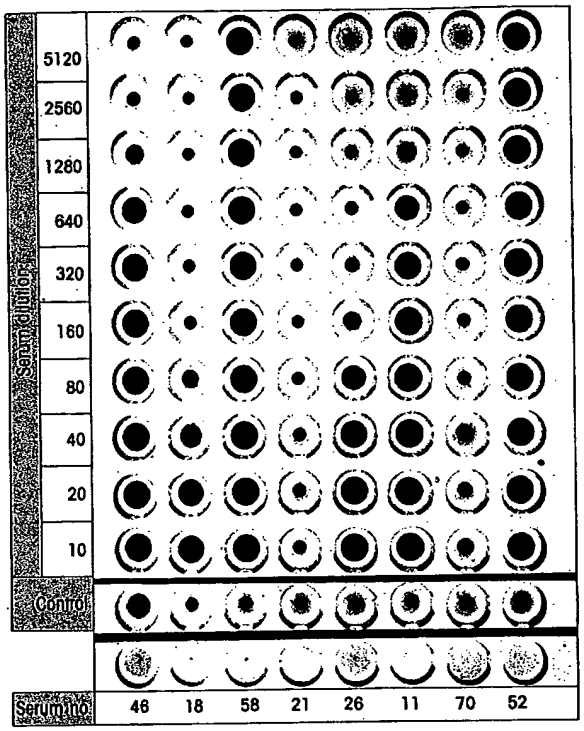

Figure 5.15. *Tanned red cell haemagglutination test for thyroglobulin autoantibodies. Thyroglobulin-coated cells were added to dilutions of patients' sera. Uncoated cells were added to a 1:10 dilution of serum as a control. In a positive reaction, the cells settle as a carpet over the bottom of the cup. Because of the 'V'-shaped cross-section of these cups, in negative reactions the cells fall into the base of the 'V' forming a small easily recognizable button. The reciprocal of the highest serum dilution giving an unequivocally positive reaction is termed the* titre. *The titres reading from left to right are: 640, 20, >5120, neg, 40, 320, neg, > 5120. The control for serum No. 46 was slightly positive and this serum should be tested again after absorption with uncoated cells.*

Purification of antigens and antibodies by affinity chromatography

The principle is simple and *very* widely applied. Antigen or antibody is bound through its free amino groups to cyanogen-bromide-activated Sepharose particles. Insolubilized antibody, for example, can be used to pull the corresponding antigen out of solution in which it is present as one component of a complex mixture, by absorption to its surface. The uninteresting garbage is washed away and the required ligand released from the affinity absorbent by disruption of the antigen–antibody bonds by changing the pH or adding chaotropic ions such as thiocyanate (figure 5.17). Likewise, an antigen immunosorbent can be used to absorb out an antibody from a mixture whence it can be purified by elution.

Immunoassay of antigen and antibody with labelled reagents

Antigen and antibody can be used for the detection of each other and an ingenious plethora of immunoassay techniques have been developed in which the final read-out of the reaction involves a reagent conjugated with an appropriate label.

A wide variety of labels is available

Radiolabelling with ^{131}I, or now more usually ^{125}I, is a tried and trusted technique with a very long history. Because of health hazards and the deterioration of reagents through radiation damage, other types of label have been sought. Enzymes such as peroxidase and phosphatase which give a coloured reaction product have been successfully employed particularly in the ELISA (enzyme-linked immunosorbent assay), an immunoradiometric assay for antibody and sometimes for antigen. One clever ploy for amplifying the phosphatase reaction is to use NADP as a substrate to generate NAD which now acts as a co-enzyme for a second enzyme system. Conjugation with the vitamin biotin is finding increasing favour since this can be readily detected by its reaction with enzyme-linked avidin to which it binds with ferocious specificity and affinity ($K = 10^{15}M^{-1}$). Other active approaches utilize chemiluminescent and new-style fluorescent tags.

Soluble phase immunoassays

For antibody

If a reasonable excess of labelled antigen is added to an antiserum, most of the antibodies of moderate affinity will be complexed and precipitation of the complexes followed by measurement of the label will give an estimate of the antigen binding capacity of the serum (figure 5.18). By using antibodies to different immunoglobulin classes and subclasses as the antiglobulin reagent, it is possible to determine the distribution of antibody activity among the classes. For example, addition of a radioactive antigen to human serum followed by a precipitating

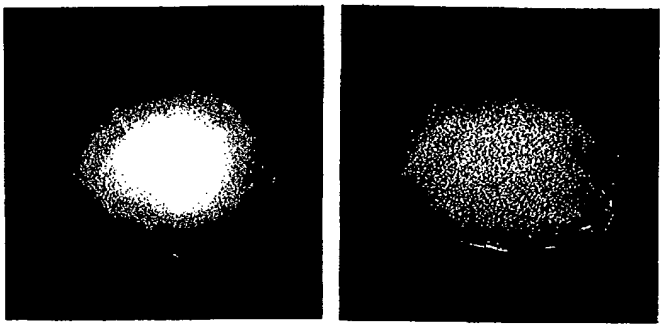

Figure 5.16. *Macroscopic agglutination of latex coated with human IgG by serum from a patient with rheumatoid arthritis. This contains rheumatoid factor, an autoantibody directed against determinants on IgG. (a) Normal serum, (b) patient's serum.*

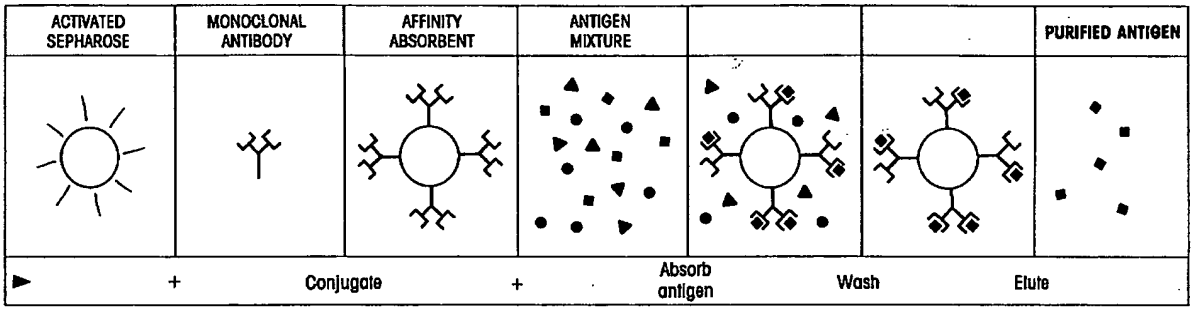

Figure 5.17. *Affinity chromatography. A column is filled with Sepharose-linked antibody. The antigen mixture is poured down the column. Only the antigen binds and is released by change in pH for example. An antigen-linked affinity column will purify antibody obviously.*

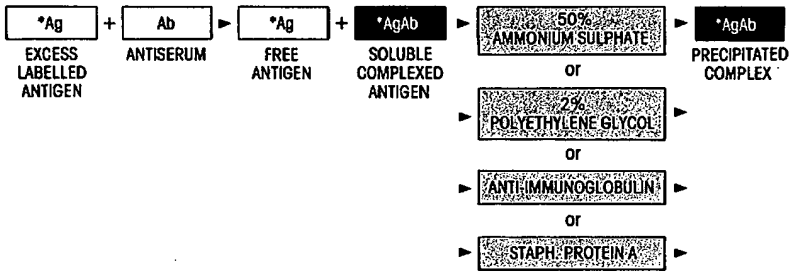

Figure 5.18. *Determination of antigen-combining capacity of an antiserum using antigen coupled to ^{125}I or some other label (cf. figure 5.12). The radioactivity of the precipitate provides a measure of the antigen-combining capacity.*

rabbit anti-human IgA would indicate how much antigen had been bound to the serum IgA.

Classical radioimmunoassay (RIA) for antigen

The binding of radioactively labelled antigen to a limited fixed amount of antibody can be partially inhibited by addition of unlabelled antigen and the extent of this inhibition can be used as a measure of the unlabelled material added. The principle of this form of saturation analysis is explained in figure 5.19. Methods vary in the means used to separate free antigen from that bound to antibody and we have discussed the main ones already.

With the development of methods for labelling antigens to a high specific activity, very low concentrations down to the 10^{-12} g/ml level can be detected and most of the protein hormones can now be assayed with this technique. One disadvantage is that these methods cannot distinguish active protein molecules from biologically inactive fragments which still retain antigenic determinants. Other applications include the assay of carcinoembryonic antigen, hepatitis B (Australia) antigen and smaller molecules such as steroids, prostaglandins and morphine-related drugs (appropriate antibodies are raised by coupling to an immunogenic carrier).